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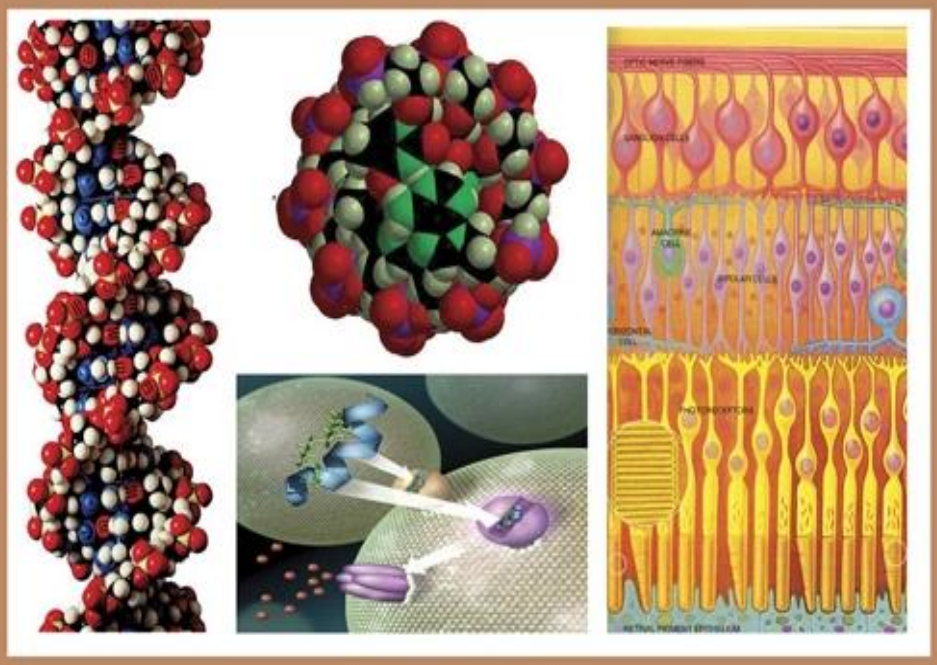

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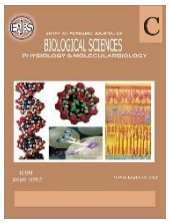

Phytochemicals and Antimicrobial Activity of *Lavandula officinalis* Leaves Against Some Pathogenic Microorganisms

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ABSTRACT

This study aims to determine the chemical composition and evaluate the antibacterial activity of the essential oil of leaves dry lavender (*Lavandula officinalis*). The analysis of the essential oil indicates the presence of terpenes, tannins, saponins, flavonoids, alkaloid, coumarins, and the absence of anthocyanins. Furthermore, of the oil's antimicrobial activity was evaluated using agar diffusion and broth microdilution methods. The antimicrobial test results showed that the oil had antimicrobial activity against all 8 microorganisms strains included in the study. Results suggest antimicrobial properties of the *Lavandula officinalis* essential oil, which may find its application in future research for the pharmaceutical industry.

INTRODUCTION

Lavandula genus (*Lamiacea*e) is a source of eco-friendly herbs because of their insecticidal activity due to their 1,8-cineole and camphor content. This genus consists of about 20 species and over 100 varieties (Quezel & Santal,1963). In Algerian flora, there are five species from which three are endemic (*Lavandula dentate, Lavadula multifida , Lavandula stoechas, lavandula officinalis and lavandula angustifolia*)Cavangh & Wilkinson, 2002). Lavender is one of the most useful medicinal plants (Boelens,1995). it is an important source of essential oils that are widely used in fragrance industry including soap, colognes, and other cosmetics (Paul *et al.*, 2004). The essential oils of Lavandula are antiseptic, relaxant anti-inflammatory and are used against headaches and scabies (Ghelardini *et al.,* 1999).

However, only in the last few years have in-depth investigations on the antimicrobial activity of lavender oil been carried out. Some studies have described its *in vitro* activity against different bacteria, including antibiotic-resistant strains and its antifungal activity, including the inhibition of conidium germination and germ tube growth for the fungus *Botrytis cinerea (*Antonov *et al*., 1997)

The antibacterial activity of Lavandula officinalis oil was investigated in this study utilizing various types of microorganisms. The composition of the phytochemicals was also determined.

MATERIALS AND METHODS

Plant material:

Samples of *Lavandula officinalis* were collected in northern Algeria (Mostaganem) in May 2022. Identification of the species was confirmed, and a voucher specimen was preserved at the university of Oran. The samples were dried and ground to a fine powder prior to analysis.

Essential Oil Isolation:

The dried aerial parts were submitted to Hydro distillation for 3 h using the Clevenger-type apparatus, according to the European Pharmacopoeia (1996). Briefly, the plant was immersed in water and heated to boiling, after which the essential oil was evaporated together with water vapor and finally collected in a condenser. The distillate was isolated and dried over anhydrous sodium sulfate. The oil was stored at 4˚C.

The yield of the plants in dry extract was determined by calculating the following ratio:

$$\textbf{Yield\%} = [P1\text{-}P2 / P3] \times 100$$

P1: Weight of the flask after evaporation, P2: Weight of the empty flask, P3: Weight of the starting dry plant material.

Phytochemicals Screening:

We conducted a phytochemical screening to gain an understanding of the major families that can be discovered in plant material. This method relies on the development of colorful or insoluble compounds. The observed coloration is usually due to the formation of conjugation or instauration in a molecule. In these characterization experiments, we use an appropriate reagent to induce this induction. We used the methods outlined by (Paris *et al.*,1969); with some changes to characterize the several chemical groups (tannin, flavonoids, sterols and steroids, alkaloids, and saponins).

Antimicrobial Activity:

The essential oils were tested on the following strains *Bacillus subtillis* ATCC9372, *Salmonella s*p, *Staphylococcus aureus* ATCC25923, *Klebsiella pneumoniae* ATCC3583, *E. coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27852, *Aspergillus niger* ATCC16404, *Candida albicans* ATCC5027. The bacterial strains used in this study were obtained from the laboratory of Experimental Biotoxicology of Biodepollution and Phytoremediation at the university of Oran Ahmed Ben Bella 1. All bacteria were stored in trypticase soy broth containing 25% (v/v) glycerol at -20°C. Prior to use, the cultures were propagated twice in the appropriate media as mentioned above to make them physiologically active.

Two techniques were used to test the antimicrobial activity of *Lavandula officinalis* oil, disk diffusion test of EOL was performed using a 24 h cultured bacteria at 37℃ in tryptic soy agar. The bacterial cultures were accustomed to 0.5 McFarland standard with sterile saline. The bacterial suspensions were spread over Mueller-Hinton agar plates using a sterile cotton swab.Sterilized paper discs (φ6 mm) were impregnated with 20 µL of EOL and placed on the inoculated agar plates. The plates were sealed with a sterile plastic wrap to avoid evaporation and incubated at 37℃ for 24 h. Finally, the diameter of inhibition zone was measured in mm. In addition to the solid medium diffusion procedure, the microplate bioassay (microdilution) was used, as recommended by NCCLS, for the determination of minimum inhibitory

concentration (MIC) (NCCLS; 1999). The MIC was defined as the lowest concentration of *Lavandula officinalis* oil inhibiting visible bacterial growth after incubation for 20h at 37°C. Into each well, 100 µL of Brain Heart Infusion broth was inoculated with the bacteria inoculum prior to the essay. An aliquot (100 µL) of the essential oil was added to the first well. Geometric dilutions ranging from 0,041 mg/mL to 21 mg/mL of the essential oils were prepared in a 96-well microtitre plate, including one growth control (BHI +Tween 80) and one sterility control (BHI+Tween 80+test oil). The contents of the wells were mixed, and microplates were incubated at 37°C for 24 h. The MIC was determined by the quantitative tetrazolium-based colorimetric method. Ten microliters of 4 mg/mL solution of 3-(4, 5-dimethylthiazo-2-yl)-2, 5-diphenyl tetrazolium bromide in distilled water were added to each well. Plates were incubated at 37°C. After a few minutes at room temperature; the plates were read. A color change from blue to purple was indicative of bacterial growth.

RESULTS AND DISCUSSION

The results obtained indicate that the extraction yield of the essential oil by hydrodistillation is 1.50 ± 0.2%. The extraction kinetics showed that almost all the essential oil is extracted after the first 90 minutes. The results obtained by (Laib and Barbat, 2011) and (Mohammadi *et al*.,2011) indicate that dry lavender flowers from two regions of Algeria have essential oil contents of 1.36% and 2.01% respectively. These variations in composition may be due to several factors, in particular, the degree of maturity of the flowers of Lavandula officinalis, the interaction with the environment (type of climate, soil), the time of harvest and the method of extraction (Botton *et al.*, 1990).

Phyto-Chemicals Analysis:

As shown in Table 1, the Phyto-chemical analysis demonstrated the presence of common phytoconstituents like Sterols and terpenes, tannins, saponins, flavonoids, alcaloïde, coumarins, and the absence of anthocyanins. Our results are consistent with those obtained by (Laib et Barbat,2011) the presence of flavonoids, tannins, coumarins, fatty acids and volatile compounds in the plant's part is an indication that the plant is of pharmacological importance (Hostettmann and Marston, 1995). This diversity of compounds could justify their use in traditional as an antiseptic agent in the cleaning of wounds, for burns and insect bites and in veterinary practice to kill lice.

Table 1: phytochemicals screening of Lavandula officinalis parasites (Meftahizade *et al*., 2011).

Compounds	**Alkaloids**	**Flavonoide**	**Tanins**	**Sterols**	**saponosides**	**Coummarin**
Observation	+	++	++	++	+	+
(-): absence ,(+) : présence in small quantity ,(++) : présence in large quantity						

Antimicrobial Activity.

Table 2 presents the inhibition zone of essential oil determined for 8 pathogens microorganisms (bacteria and fungi) using the diffusion technique on solid media and the microdilution assay. The results showed that the essential oil had a substantial inhibitory effect on all assayed bacterial and fungal strains noted by large growth inhibition halos. The data indicated that Gram-positive *Staphylococcus aureus* was the most sensitive strain tested to the oil of *Lavandula officinalis* with the strongest inhibition zone (23,7 mm). *E. coli* was found to be more sensitive among Gram-negative bacteria with an inhibition zone of 20 mm. The oil also exhibited high antimicrobial activity against *klebsiella pneumoniae* and *candida.* Modest activities were observed against *pseudomonas aeruginosa* as well as fungi *Aeprgillus niger* with inhibition zones of 10,5 mm and 10,7 respectively.

Table 2: Antimicrobial activity of *Lavandula officinalis*

Microorganisms	Essential oil zone inhibition(mm)
Bacillus subtillis ATCC9372	16,3
Salmonella sp	19
Staphylococcus aureus ATCC25923	23,7
Klebsiella pneumoniae ATCC3583	18
E. coli ATCC25922	20
Pseudomonas aeruginosa ATCC27852	10,5
Aspergillus niger ATCC16404	10,7
Candida albicans ATCC5027	15,5

The results of the MIC are presented in Table 3. The data indicate that the oil exhibited varying levels of antimicrobial activity against the investigated pathogens strain. MIC values showed by the essential oil were in the range of 0.041 to 10 mg/mL. In a liquid medium, the essential oil was active against all the test strains. The Gram-negative *Pseudomonas aeruginosa* seemed to be resistant to the investigated oil with a MIC of 10 mg/mL. Maximum activity was observed against the *Staphylococcus aureus* with a MIC of 0.041 mg/mL. *E. coli* was the least sensitive bacteria with a MIC of 0.167. *Aspergillus niger* and *Candida albicans* showed similar susceptibility to the investigated oil, ranging from 0,084 mg/mL.

Several studies (Sandri *et al.*, 2007; Zarai *et al.*, 2011; Al-Bayati, 2008) have reported that Gram-positive bacteria are more susceptible to essential oils than Gram-negative attributed to the presence of an outer membrane, impermeable to hydrophobic compounds owing to its lipopolysaccharide coating. The absence of this barrier, in Gram-positive bacteria, allows direct contact of the hydrophobic constituents of the essential oil with the phospholipid bilayer of the cell membrane, thus causing either an increase in the permeability of ions and the escape of vital intracellular constituents causing a deficiency in the enzymatic system (Sandri *et al.*, 2007; Zarai *et al.*, 2011).

Table 3: Minimal inhibitory concentration (MIC) of essential oil from Lavandula dentata

Microorganisms	Essential oil MIC (mg/ml)
Bacillus subtillis ATCC9372	0,021
Salmonella sp	0,039
Staphylococcus aureus ATCC25923	0,041
Klebsiella pneumoniae ATCC3583	0,038
E. coli ATCC25922	0,167
Pseudomonas aeruginosa ATCC27852	10
Aspergillus niger ATCC16404	0,084
Candida albicans ATCC5027	0,084

Our findings concur with those of Chahboun et al. (2015) and Jianu et al. (2013), who found that Staphylococcus aureus was very sensitive to lavender essential oil.

CONCLUSION:

This work aimed to determine the yield, phytochemical composition, and antimicrobial properties of the essential oil of lavender grown in the region of

Mostaganem in northern Algeria. The results obtained indicate that the extraction yield of the essential oil by hydro distillation is 1.50 ± 0.2%. The analysis of the chemical composition indicates the presence of terpenes, tannins, saponins, flavonoids, alkaloid, and coumarins. This variety of compounds could support their traditional use as an antiseptic agent in cleaning wounds, for burns and insect bites, and in veterinary practice to kill lice and other animal parasites.

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